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The impact of the addition of humic substances on growth efficiency, excretion parameters and fecal microbiota in piglets

Vplyv prídavku humínových látok u mladých prasiat na rast, parametre exkrécie a mikrobiológiu trusu

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ABSTRACT

The aim of this research was to investigate the influence of dietary humic substances on growth efficiency, faeces indicators, and the bacteriological composition of faeces in piglets. Twenty-four crossbred young pigs (Slovakian White x Landrace) in total were allotted into two groups: the experimental and the control groups. They were fed a diet with humic substances supplemented at 0.5% in the experimental group. In this study, there were no significant differences in the monitored production indicators of piglets. The addition of humic substances increased the dry matter of faeces in the experimental group ($P < 0.001$). Other indicators in the faeces samples were not significantly affected by dietary treatment. We observed a significant decrease in the counts of coliform bacteria in the faeces ($P < 0.05$) of the experimental group. The results also showed a tendency to increase the counts of the lactic acid bacteria and a decrease in the counts of the Enterobacteriaceae in the piglets' experimental group compared to the control group. A decrease in coliform bacteria counts in the faeces of experimental animals showed a positive effect on the microbiota in the intestine. It was concluded that the addition of humic substances to the diet of piglets could have a positive impact on gut flora without having a negative effect on production performance or other fecal characteristics.

Keywords: humic substances, faeces, microbiota, productive performance, piglets

ABSTRAKT

Cieľom tejto štúdie bolo hodnotiť vplyv humínových látok v krmive na rastovú výkonnosť, vlastnosti trusu a bakteriologické zloženie vzoriek trusu u prasiatok. Celkom 24 krížencov (Slovenská biela × Landrace) bolo rozdelených do dvoch skupín: kontrolná skupina a experimentálna skupina. Boli kŕmené kŕmnou zmesou doplnenou o prídavok humínových látok v množstve 0,5% v experimentálnej skupine. V tejto štúdii neboli zistené signifikantné rozdiely v sledovaných produkčných ukazovateľoch prasiatok (telesná hmotnosť, prírastok telesnej hmotnosti a konverzia krmiva). Suplementácia humínových látok zvýšila obsah sušiny v truse v porovnaní v experimentálnej skupine ($P < 0,001$). Ostatné sledované parametre vo vzorkách trusu neboli významne ovplyvnené príjmom krmiva s prídavkom humínových látok. Pozorovali sme významný pokles počtu koliformných baktérií vo výkaloch ($P < 0,05$) experimentálnej skupiny. Výsledky tiež ukázali tendenciu zvyšovania počtu baktérií mliečneho kvasenia a poklesu počtu Enterobacteriaceae v experimentálnej skupine prasiatok v porovnaní s kontrolnou skupinou. Pokles počtu koliformných baktérií vo výkaloch pokusných zvierat preukázal pozitívny vplyv na črevnú mikroflóru. Dospelo sa k záveru, že prídavok humínových látok do krmiva prasiatok by mohol mať pozitívny vplyv na črevnú flóru bez negatívneho vplyvu na produkčné parametre alebo iné vlastnosti trusu.

Kľúčové slová: humínové látky, výkaly, microbiota, produkcia, mladé ošípané

INTRODUCTION

Feed additives are currently successfully and widely applied in animal nutrition. The biological and chemical breakdown of plant biomass by microbes results in the formation of humic substances (HS), which are naturally occurring organic bioactive compounds present in soil (Marcinčák et al., 2023). These organic residues, which contain humic and fulvic acids, are made up of decomposing organic materials.

By EU law, antibiotics used as growth boosters are prohibited because of the spread of bacterial resistance (Islam et al., 2005). HS as an alternative to growth promoter antibiotics is used for its inflammatory-reducing, adsorbent, and antibacterial properties (Kühnert et al., 1991), which makes it suitable to be successfully used in veterinary medicine.

HS are currently being tested in a number of animal feeding fields (Bezuglova and Klimenko, 2022). They, in various forms and concentrations, have been relatively successfully applied in numerous experiments with poultry (Bailey et al., 1996; Kocabağli et al., 2002; Marcinčáková et al., 2015; Arif et al., 2019; Semjon et al., 2020).

It was previously proposed that HS regulate the intestinal flora, ensure greater nutrient utilization in animals, and boost the beneficial bacteria while suppressing the harmful ones within the body (Islam et al., 2005). Recent findings in pigs and broiler chickens appear to support this assertion (Visscher et al., 2019; Mudroňová et al., 2020).

The use of HS in pig feed has yet to be thoroughly researched (Kim et al., 2004; Kunave and Lien, 2012). The ability of humic compounds to prevent intestinal illnesses and promote pig growth has sparked an increase in interest in their usage as feed additives in recent years (Trckova et al., 2005; Ponce et al., 2016). According to Heo et al. (2013) and Rist et al. (2013), digestive problems with diarrhea are common during the weaning period and the post-weaning period in piglets. Improvements in body weight, weight gain, feed intake, and feed conversion ratio were seen in weaned pigs who received HS supplements (Kim et al., 2004; Trckova et al., 2018; Dell'Anno et al., 2020; Wang et al., 2020). Less severe diarrhea was seen in pigs with HS added (Kaevska et al., 2016; Trckova et al., 2018).

Since there aren't many studies on the intestinal microbiota of pigs, the goal of this study was to find out how adding humic chemicals affected the fecal microbiota, other chosen faeces properties, and piglet growth.

MATERIALS AND METHODS

Experimental Design

Two groups (n = 12 each) comprising a total of 24 crossbred (Slovakian White x Landrace) 35-day-old piglets were created. Piglets were weighed before and after the experiment to determine the average daily rise in body weight. The daily feed intake in both groups was recorded. The feed conversion ratio was determined as the ratio between feed intake and body weight gain. The experimental group, whose diet included a 0.5% humic substance (HS) supplement, and the control group were both included in the study. A 28-day experiment was conducted. In the experiment, both groups had the same diets. Diets were composed of cereal grains (maize, wheat, and barley), soybean meal, and mineral-vitamin-amino acid premix. The elimination of barley grain allowed for the insertion of the HS supplement into the experimental diet. The analysis of feed mixtures for piglets in both groups is shown in Table 1.

Drinking water and feed mixtures were available to the piglets *ad libitum*. The HS supplement contained a mixture of humic acids and fulvic acids (min. 65% and 5% in dry matter, respectively).

This study was approved by the Ethics committee of the UVMP in Košice (2022-11).

Laboratory analysis

The diets were analysed for nutritional composition (dry matter, crude protein, crude fibre, total ash, starch, and total phosphorus) according to EC Commission

Regulation 152/2009. The diet samples were dried at 105 °C and ashed at 550 °C for determination of the dry matter and the total ash content. The crude protein was determined using the Kjeldahl method, and the content was calculated by multiplying the nitrogen value by a coefficient of 6.25. Crude fibre was determined by defatting the well-dried samples and separating the residue in a Dosi-Fibre extractor (J.P. Selecta S.A., Spain). The starch content of diets was determined polarimetrically, and the total dietary phosphorus was determined using the photometric method. The level of dietary minerals (calcium and sodium) was analysed using the atomic absorption spectrometer (Unicam AAS spectrometer 939, United Kingdom). The metabolizable energy values of diets were computed according to the Šimeček, Zeman and Heger (2000).

A sampling of faeces for microbiological analysis as well as for evaluation of selected excretion parameters was performed on animals individually 4 hours after the morning feeding on the last day of the experiment.

1 g of faeces was homogenized (Stomacher Lab Blender 80, Seward Medical Limited, Great Britain) with 9 ml of saline. Samples were diluted by decimal dilution. The following media were used: MRS agar (Merck) for lactic acid-producing bacteria; MacConkey agar (Oxoid Unipath) for coliform bacteria; M-Enterococcus agar for enterococci (Becton Dickinson); and Endo agar (Oxoid Unipath) for enterobacteria. Nutrient media for determining the numbers of facultative anaerobic bacteria were incubated for 48 hours at 37 °C under anaerobic conditions (Gas Pak Plus, BBL, Microbiology Systems, Cockeysville, USA). After incubation, bacterial counts were determined as log10 KTJ/g faeces.

Analysis of individual parameters of excretion: dry matter by drying under standard conditions; crude protein (from fresh matter)—for the Kjeltec device (Kjeltec 2300, Foss Tecator, Sweden); short-chain fatty acids (SCFA)—isotachophoretic determination on a two-column analyzer EA100 (Villa Labeco, Slovak Republic); pH—injection combined glass electrode with polymer electrolyte, suitable for measuring solid samples.

Table 1. Analysis of feed mixtures for piglets (in the original dry matter)

		Control diet	Experimental diet
DM	(%)	88.5	88.4
CP	(%)	17.8	17.7
CFi	(%)	3.54	3.89
Total ash	(%)	5.41	5.76
Starch	(%)	44.5	43.8
Ca	(%)	0.64	0.7
P	(%)	0.56	0.56
Na	(%)	0.27	0.28
Metabolizable energy	(MJ/kg)	13.07	13.02

DM – Dry matter, CP – crude protein, CFi – crude fibre, Ca – calcium, P – Phosphorus, Na – sodium

Statistical analysis

The statistical evaluations of the influence of HS on production and faeces characteristics were done by an unpaired T-test (statistical software GraphPad Prism 8.0). The values of $P < 0.05$, $P < 0.01$, and $P < 0.001$ were considered statistically significant.

RESULTS

Growth performance

Some breeds, such as Slovakian White, Landrace, and especially their hybrids in various combinations, can be characterized by special traits that lead to better growth performance. The effects of the dietary HS treatments on growth performance (final weights, daily gains, feed intake, feed conversion ratio) are presented in Table 2. There were no significant differences in piglets fed diet with the 0.5% HS compared with those receiving the control diet. As regards the average daily feed intake, it was balanced in both groups. The insignificantly better feed conversion ratio was observed due to the more intense growth of piglets in the experimental group.

Table 2. Effects of HS on growth performance in piglets

		Control group x ± SD	Experimental group x ± SD
Start B.W.	(kg)	11.68 ± 1.90	11.65 ± 1.90
Final B.W.	(kg)	29.02 ± 3.49	29.57 ± 3.60
ADG	(kg)	0.619 ± 0.07	0.640 ± 0.07
ADFI	(kg/group)	1.127	1.126
Feed conversion ratio	(kg/kg/group)	1.82	1.76

x – average value, SD – standard deviation, B.W. – body weight, ADG - Average daily gain, ADFI - Average daily feed intake, FCR - Feed conversion ratio

Faeces parameters

The analysis of selected excretion indicators and fermentation products by examination of fresh faeces samples collected from animals individually in both groups is summarized in Table 3.

In the experimental group, a significantly higher percentage of faeces dry matter was analyzed compared to the control group ($P < 0.001$). The rest of the evaluated excretion parameters (crude protein content) and fermentation parameters (short-chain fatty acids) were not statistically significantly affected compared to the control group, although in the faeces of the control animals, the concentrations of acetate, propionate, and butyrate were higher compared to the experimental group. Also, the total short-chain fatty acid (SCFA) content in faeces was higher in the control group (plus 11.57 mmol/kg compared to the experimental group).

Fecal microbiota

The presence of enterococci, lactic acid bacteria, coliform bacteria, and Enterobacteriaceae was used to measure the impact of humic substance addition on the microflora in the faeces. Colony-forming units per gram of content ($\log_{10}$ cfu/g) were used to express the bacterial numbers. While the numbers of coliform bacteria reflect various enteropathogens, lactic acid bacteria represent the beneficial microbiota. Despite the fact that there was no statistically significant difference in the number of lactic acid bacteria between the experimental and control groups, the number of coliform bacteria was considerably lower in the faeces samples ($P < 0.05$) (Table 4).

Table 3. Analysis of the fecal parameters and fermentation products

Parameter		Control group x ± SD	Experimental group x ± SD
DM	(g/kg fresh matter)	221.9 ± 6.9	246.6 ± 3.3 ***
CP	(g/kg DM)	211.0 ± 13.0	198.0 ± 14.6
Acetate	(mmol/kg fresh matter)	91.39 ± 7.35	84.52 ± 8.27
Propionate		42.82 ± 6.21	39.21 ± 5.88
Butyrate		19.58 ± 4.63	18.49 ± 3.66
Total SCFA		153.79 ± 18.19	142.22 ± 17.81

x – average value, SD – standard deviation, DM – dry matter, CP – crude protein, SCFA – Short-chain fatty acids (acetate + propionate + butyrate); *** – $P < 0.001$ – statistical significance compared to the control sample (unpaired *T*-test)

Table 4. Bacteriological examination of faecal samples

	Control group x ± SD	Experimental group x ± SD
Enterococci	6.66 ± 0.47	6.60 ± 0.40
Lactic acid bacteria	6.30 ± 0.36	6.53 ± 0.40
Coliform bacteria	6.35 ± 0.31	5.85 ± 0.29 *
Enterobacteriaceae	6.55 ± 0.40	6.35 ± 0.42

x – average value, SD – standard deviation; * – $P < 0.05$ – statistical significance compared to the control sample (unpaired *T*-test)

DISCUSSION

For many reasons, humic substances (HS) attract the increased attention of scientists. Depending on the region of occurrence, heterogeneous macromolecular structures and compositions of HS may change (Marcinčák et al., 2023). Because of their disparate origins and natures, as well as the fact that their bio-effects vary depending on specification, it is therefore challenging to compare the real effects of HS preparations. By raising animal growth rates, increasing feed efficiency, reducing digestive disorders, and boosting general immunity, humic compounds are utilized as feed supplements in animal husbandry to improve the economics and ecology of animal production (Goel and Dhingra, 2021).

Dietary HS can either have no effect (Weber et al., 2014; Ponce et al., 2016) or boost the average daily weight gain in young pigs (Wang et al., 2008). The findings of the Ji et al. (2006) study show that adding humic acids to pig diets to enhance growth metrics has a variety of impacts. Humic compounds as feed additives in pigs have also been demonstrated to have the potential to enhance growth intensity (Hristov et al., 2009). The measured piglet production parameters (final body weight, average daily growth, and feed conversion ratio) by HS dietary treatment were not significantly different in this study.

According to some authors (Ji et al., 2006; Wang et al., 2008), growing pigs fed diets containing HS showed a larger average daily gain, but average daily feed intake was unaffected. However, Trckova et al. (2006) found no difference in the growth performance of pigs fed diets supplemented with HS. Further research is necessary to determine the cause of the disparity in growth performance. Wang et al. (2008) reported that long-term administration of HS may have a more noticeable effect. The effects of HS may also be related to the length of administration. The uneven outcomes could be primarily attributed to the numerous animal species, groups, and ages used in various trials, as well as the variable composition of various HS preparations and addition levels.

Zraly and Pisarikova's (2010) study showed that humic acid salts, such as sodium humate, can enhance the growth efficiency of piglets fed diets containing 1% sodium humate. In Kaevska et al.'s (2016) study, supplementing zinc oxide (ZnO) with sodium humate (20 g/kg) in feed did not substantially alter body weight increase (g/day) or feed intake (g/pig/day) when compared to the group that received ZnO alone for the duration of the experiment. The results of Wang et al. (2020) demonstrated that adding sodium humate to the food improves the growth rate in weaned piglets.

Flohr et al. (2012) did not see any appreciable changes in the dry matter of faeces in pigs receiving a 1% mixture of humic and fulvic acids, in contrast to our work. The total short-chain fatty acid (SCFA) concentration was in line with data from the literature. The overall SCFA concentrations in the animals were greater in the study by Metzler-Zebeli et al. (2011) with weaned piglets (160.8 and 182 mmol/kg fresh matter, respectively) than in the current investigation. Our findings corroborate those of Visscher et al. (2019), who discovered that the inclusion of HS in complete diets typically led to reduced SCFA contents, namely lower concentrations of acetic and propionic acid and hence lower total SCFA contents.

In this investigation, however, the variations in total and individual SCFA concentrations across groups were not statistically significant.

There is currently very little known about the impact of humic compounds on the microbiology of the faeces and the health of the intestines. According to Shermer et al. (1998), adding humic compounds to feed can encourage greater Lactobacillus populations in broilers' caecums.

Only a minor portion of the microflora in faeces is made up of lactobacilli (Pajarillo et al. 2014).

The impact of humic compounds on coliform bacteria counts in pigs is currently poorly understood. We found a substantial decrease in coliform bacteria in the faeces contents during the research on the impact of HS on the intestinal microbiota ($P < 0.05$), but no significant rise in lactic acid bacteria counts.

Chickens, however, have yielded some contradictory results (Shermer et al., 1998; Aksu and Bozkurt, 2009). After HS administration, lactobacilli counts only slightly increased but E. coli levels in the broiler's intestinal contents significantly decreased, according to Aksu and Bozkurt's 2009 findings. On the other hand, Shermer et al. (1998) found no significant changes in the caecal microflora when chicks were given a lower concentration of humates (0.5%) but a significant increase in the number of E. coli in the caecum of chickens after administration of higher humate concentrations (1% and 5%, respectively).

CONCLUSION

In conclusion, we found that 0.5% HS had a favorable impact on the microbiota in faeces. Animals in the experimental group had lower levels of coliform bacteria in their faeces, indicating that there was a favorable impact on the gut microbiota. The other faecal features, however, were unaffected. In this investigation, HS-supplemented diets for piglets had no discernible impact on their ability to grow.

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